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MIXED INFECTION WITH AEROSOL OF VIRUSES OF  
ORNITHOSIS AND INFLUENZA

- USSR -

[Following is a translation of an article by  
I. I. Terskikh and A. I. Gromyko in the Russian-  
language publication Voprosy Virusologii (Pro-  
blems of Virology), No 1, 1966, pages 60-84.]

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Submitted on 28 October 1963

Experimental study of mixed aerosol infection with viruses  
of ornithosis and influenza has defined practical value and presents  
great theoretical interest. In the period of outbreaks of influenza  
there is the possibility of the appearance of infection mixed with  
ornithosis in the first place at bird-processing production lines,  
in bird farms, and also in the middle of the population contacting  
pigeons and other birds. Diagnostics and the flow of such mixed  
infection, as shown by results of our experiments, have their own  
peculiarities. In the literature we did not find data on the study  
of mixed infections with viruses of ornithosis and influenza or  
general indications of the stimulating role of the virus of influ-  
enza in mixed infections.

In this report we give data of an experimental study of  
mixed infection induced in animals in various sequences of infection  
with the aerosol of viruses of ornithosis and influenza.

Material and Method

Use was made of the virus of ornithosis adapted to the lungs  
of white mice (the psittacosis strain Lori) and the virus of in-  
fluenza (strain PR-8 of type A).

Accumulation of the virus of ornithosis in organs of the

experimental animals was determined by titrating on white means by means of infection in the brain (the LD<sub>50</sub> was determined after Read and Munch).

The virus of influenza was determined by the RRH [reaction of retarded hemagglutination] and also from the infectional titer in the lungs of white mice.

Animals were infected with the aerosol in the IVK-2 chamber (See Note). White mice weighing 7-8 grams each were placed in a special cartridges guaranteeing the hit of the virus only through the organs of breathing.

(NOTE) The design was developed by I. I. Terskikh, V. M. Lolotov Bolotovskiy, and I. V. Kashin (Voprosy virusologii, 1961, No 6, pages 743-745).

The virus-containing suspension was dispersed by an atomizer creating an aerosol, 90 percent of the particles of which had a diameter of 1-3 microns.

After development of the aerosol cloud, the animals during an hour were found in the chamber (contact). The relative humidity in the chamber was around 85 percent, the temperature -- 20-21 degrees. The volume of the working chambers was 220 liters, and the rate of air supply (in the aerosol) was 30 liters/minute. Full "saturation" of the chamber with the contagious aerosol occurring in 8 minutes. Under such conditions of the experiment, every mouse could obtain 0.004 ml of the virus-containing material in the aerosol. Simultaneously studied was mixed infection and monoinfection, that is, only with the virus of ornithosis and only the virus of influenza. The criterion of appraisal was the death of the animals, the dynamics of lesions in the lungs and other organs, established through histological examination of sections.

### Results

Figure 1 gives the total data which show that during simultaneous infection with two viruses, already in 48 hours in the lungs of white mice the virus of ornithosis was stored in high enough titers, but significant titers of the virus of ornithosis in the controls will appear only from the sixth day following infection. In the use of a still smaller dose of the virus of ornithosis -- 0.1 LD<sub>50</sub> -- the intense accumulation of this virus in the presence of the influenza virus more clearly is disclosed (Table 1).

It is necessary to note that in the case of a dose of virus of influence of 1 LD<sub>50</sub>, which was used for reproduction of the mixed infection, the virus of influenza could not be disclosed with the help of the RRH neither in cases of mixed infection nor in the controls. By carrying out one passage in the allantoic cavity of chick embryos of the suspension of the lungs of white mice it was also not possible to reveal the virus of influenza

with the help of hemagglutination. The latter can be disclosed by this method only in cases when the influenza infection was reproduced with large concentrations of virus ( $100 \text{ LD}_{50}$ ) or biologically in passages in white mice and embryos.

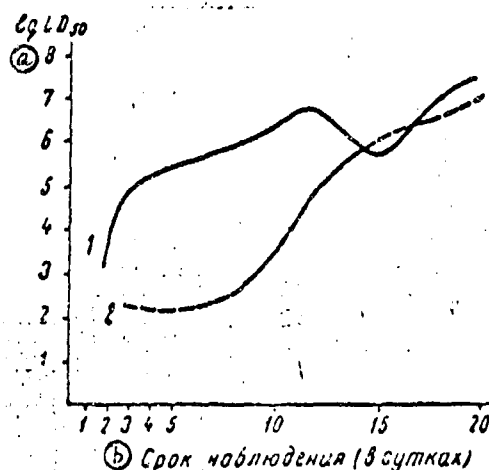


Fig. 1. Dynamics of accumulation of the virus of ornithosis in the lungs of white mice in mixed infection. 1 -- mixed infection; 2 -- controls. LEGEND: a)  $\lg \text{LD}_{50}$ ; b) period of observation (in twenty-four hour days).

Table 2 gives data on the manifestation of the hemagglutinating properties of the virus of influenza in the lungs of white mice in infection with various doses of the aerosol of virus.

For a study of the role of the virus of influenza in mixed aerosol infection, one  $\text{LD}_{50}$  of the virus of influenza was stratified after 48 hours of infection with the virus of ornithosis (Figure 2).

As can be seen from Fig. 2, in spite of later penetration of the virus of influenza in the cell, accumulation of the virus of ornithosis in the lungs of white mice is also stimulated. Thus, if in the controls the titer of the virus of ornithosis attains the greatest value by the 12th day, then in such mixed infection the virus is disclosed in significant titer already from the fifth day, that is, somewhat later than in simultaneous introduction.

Thus, the presence of the virus of influenza as it were stimulates reproduction of the virus of ornithosis. The mechanism of this process is meanwhile vague: whether in the beginning the virus of influenza renders its damaging action on the cell or whether this is connected with double virus information. These questions are being studied.

TABLE 1

Dynamics of Accumulation of Virus of Ornithosis in Mixed Infection with Minimal Doses of the Virus of Ornithosis ( $0.1 \text{ LD}_{50}$ ) and the Virus of Influenza ( $1 \text{ LD}_{50}$ )

a) Интервал времени	b) Титр вируса орнитоза в легких выживших животных	
	в) при смешанной инфекции	г) при инфекции вирусом орнитоза
24 часа e)	1.75	2.00
3 дня f)	3.25	1.50
6 дней g)	6.00	0
9 "	5.50	0
12 "	Выше 5.50	0
15 "	6.00	0
20 "	7.50	0

LEGEND: a) interval of time; b) titer of the virus of ornithosis in the lungs of surviving animals; c) in mixed infection; d) in infection with the virus of ornithosis; e) hours; f) days; g) above.

TABLE 2

Manifestation of the Hemagglutinating Properties of the Virus of Influenza in the Lungs of White Mice in Aerosol Infection with Various Doses of Virus

a) Интервал времени	1 $\text{LD}_{50}$	100 $\text{LD}_{50}$
	b) титр в РГА	
48 часов d)	0	1:640
3 дня e)	0	1:320
4 "	0	1:640
5 дней e)	0	1:320 c)
6 "	0	Не исследовали
9 "	0	"
12 "	0	"
15 "	0	"
20 "	0	"

LEGEND: a) time interval; b) titer in RRH; c) did not investigate; d) hours; e) days.

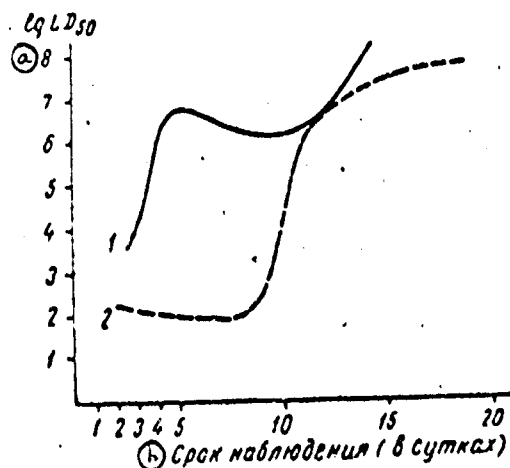


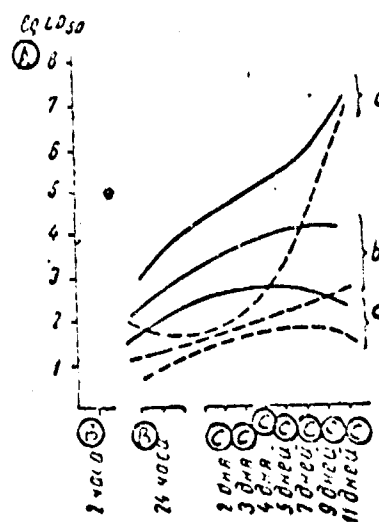
Fig. 2. Dynamics of accumulation of the virus of ornithosis in the lungs of white mice in infection with a small dose of the aerosol of the virus of influenza ( $1 \text{ LD}_{50}$ ) in 48 hours after infection with the virus of ornithosis. 1 -- mixed infection; 2 -- control. LEGEND: a)  $\lg \text{LD}_{50}$ ; b) period of observation (in twenty-four hour days).

More rapidly and at a higher titer, the accumulation of the virus of ornithosis induces in animals also a graver clinical manifestation of infection at high fatality. Morphologically, in the lungs a more intense lesion is noted in the form of confluent hemorrhagic pneumonia. Specific diagnostics of ornithosis when mixed with influenza infection does not present difficulties, however one did not manage to disclose influenza infection. Whether the virus of influenza plays a role only in the beginning of the process, and then its reproduction is suppressed as a result of intense accumulation of the virus of ornithosis, or whether we did not succeed in disclosing small doses in the mixture culture -- remains unclear.

However, the obtained data permits making the following conclusion. In the development of a mixed aerosol infection, the use of excitants (pneumotropics) with the identical mechanism of penetration has value. Here, the virus of influenza presents definite interest. Thus, the speed of reproduction (in the cell) of the virus of influenza penetrates significantly faster and is developed in a sensitive cell, as if preparing it for subsequent penetration and development of the virus of ornithosis. By this it is possible to explain the significant strengthening of the pathological process in the organism of animals in mixed infection.

The dynamics of accumulation of the virus of ornithosis in the lungs is disclosed and corresponds to the dynamics of lesions in the organs of animals (in histological research). Thus, in infection only with the virus of ornithosis (a dose of 0.1 LD<sub>50</sub>), inflammatory changes in the lungs of the animals are possible to be noted by the third day, the greatest dissemination of the process -- in seven days, and then the animal recovers.

Figure 3. Titers of the virus of ornithosis in the organs of white mice in mixed infection (solid line -- mixed infection, dashed line -- control). a -- lungs; b -- liver and spleen; c -- blood. LEGEND: A) lg LD<sub>50</sub>; B) hours; C) days.



In mixed infection, even in 18 hours swelling of the bronchial epithelium and separate extravasates are noted. Changes are lacking in the parenchymatose organs. In 24 hours one may see lesions of the small bronchi with exudates, but in pulmonary tissue small foci of infiltration with polynuclearites is observed. In 30-36 hours, lesions of the lungs are noted more characteristic for ornithosis, as well as focal alveolitis and infiltration with polynuclearites. By the fifth - seventh day, confluent foci of pneumonia are revealed, especially in the marginal sections. On the basis of a comparative study of the character of lesions in the organs of animals infected in various sequences with the virus of influenza and of ornithosis, it is possible to assume that in the first 18 hours lesions of pulmonary tissue is caused by the virus of influenza, and the subsequent development of the process (rapid and graver) -- by the dual infection, but mainly by the virus of ornithosis. This is confirmed also by the higher titer of the virus of ornithosis in the lungs in mixed infection. Lesions of parenchymatose organs is caused by the virus of ornithosis, the higher titer of which is fixed in mixed infection not only in the blood, but also in the parenchymatose organs, that is, the development of infection occurs on the type of septicemia (Figure 3).

The mechanism of the more active development of ornithosis in the presence of the virus of influenza is not quite clear and will be studied on isolated cells (culture of tissue) with the use of the method of immunofluorescence and other methods. However, the obtained data permits noting that for reproduction of grave aerosol infection combination of excitants with a common mechanism of penetration and a common tropism to tissue is necessary, but which have different speeds of reproduction. As a result, "stratification" occurs and the strengthening of the pathological process. Such interrelations in the development of mixed infection one can manage to disclose through the use of small, subcontagious doses of excitants. The definite medical effect will be attained already after suppression of development of one of these excitants, but in early periods after the infection.

Study of interrelations between various excitants in the cell and organism presents the greatest interest and is promising for the solution of questions of diagnostics and therapy of mixed infections. These investigations will help to open up the "mechanism" of development of mixed infections, usually determining the heavier flow of disease and not infrequently fatal outcomes.

#### Conclusions

1. In infection with subcontagious doses of the virus of influenza and ornithosis (in aerosol) in animals, a heavy flow of mixed infection is observed.

2. A subcontagious dose of the virus of influenza stimulates the development of ornithosis in animals with simultaneous or consecutive infection.

3. In the presence of the virus of influenza, more intense accumulation of virus of ornithosis in the tissues of animals is accompanied by widespread lesions in organs: in the lungs, then in the liver, and the spleen.

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